International Journal of Food Microbiology 129 (2009) 93–98

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Short Communication

# Characterization of *Salmonella* isolates from retail foods based on serotyping, pulse field gel electrophoresis, antibiotic resistance and other phenotypic properties

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ARTICLE INFO

*Article history:*
Received 14 August 2008
Received in revised form 28 October 2008
Accepted 2 November 2008

*Keywords:*
*Salmonella*
Pulse field gel electrophoresis (PFGE)
DNA fingerprinting
Food-borne pathogens

ABSTRACT

Sixteen *Salmonella* strains isolated from a variety of foods during 2000 and 2003 by the Florida State Department of Agriculture were characterized by various genotypic and phenotypic tests. Among 16 isolates, 15 different serotypes were identified. Pulse-field gel electrophoresis (PFGE) fingerprinting profiles obtained using restriction endonucleases XbaI and BlnI revealed that 16 *Salmonella* isolates were genetically diverse with 16 unique PFGE patterns. The PFGE pattern of eight isolates matched with the CDC/FDA data base of previous outbreaks and clinical isolates indicating their potential to cause disease. With the exception of isolates obtained from alligator meat (tetracycline resistant) and orange juice (chloramphenicol and sulfisoxazole resistant), the remainder of the isolates were susceptible to the panel of 15 antimicrobials tested. Molecular subtyping was further complimented by a variety of phenotypic tests such as acid-tolerance, Caco-2 cell invasion and biofilm formation which have often been used as a gauge of virulence and infection potential of *Salmonella* isolates. The induced acid tolerance level of the isolate obtained from orange juice was not significantly different from the laboratory reference strain *S. enterica* serovar Typhimurium SL1344. Six isolates exhibited very low levels of constitutive acid-tolerance, of which four isolates failed to infect differentiated Caco-2 cells. Although all isolates formed biofilms, there was no clear relation between the ability to form biofilms, infect differentiated Caco-2 cells and induce acid-tolerance. This study indicated that different serotypes of *Salmonella* were present in a variety of retail foods and exhibited diverse phenotypic characteristics.

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## 1. Introduction

Infections by *Salmonella* serovars is a major health concern world wide and it is estimated that 95% of these infections are food-borne. Notyphoidal salmonellosis is usually acquired by ingestion of contaminated water or food, and poultry products are a major source in many developed countries (Hald et al., 2004). The number of cases based on recent FoodNet data (CDC, 2005) is 147 per million persons, with 40% of those cases occurring in children under 15 years old.

Pulse field gel electrophoresis (PFGE) profiling is a DNA fingerprinting method which is based on the restriction digestion of purified genomic DNA. It is currently considered the gold-standard method for subtyping food-borne pathogens (Whittam and Bergholz, 2007). PFGE forms the basis for PulseNet, a national molecular subtyping network that was established in 1996 by the Center for Disease Control (CDC) and is now utilized by all state public health laboratories and food safety laboratories at the Food and Drugs Administration (FDA) and the United States Department of Agriculture (USDA). Currently PFGE data are considered reliable and a sensitive way to detect differences between closely related strains, so that isolates with indistinguishable PFGE profiles can be classified as epidemiologically linked with a high degree of confidence (Whittam and Bergholz, 2007). The broad applicability and informativeness of PFGE, however, is limited since it does not offer phenotypic characterization of pathogens. Our knowledge on routes of foodborne transmission of *Salmonella* has been acquired mostly through the study of epidemiological data from various prevalence studies and outbreak investigations. It is important to carryout phenotypic characterization of strains isolated in outbreak as well as sporadic cases since bacteria can change with or without altering the PFGE pattern (Shen et al., 2006) and thereby contributing to loss of information about the history of divergence.

In order to expand the advantages of PFGE beyond outbreak investigations we analyzed *Salmonella* isolates obtained by the Florida State Department of Agriculture through their routine survey over the period of 2000 and 2003. We reasoned that molecular subtyping in combination with phenotypic characterization will contribute

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0168-1605/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.ijfoodmicro.2008.11.007

towards quantifying the various sources of hazards and risks from sporadic occurrence of *Salmonella* strains. The four main objectives of this study were (i) to determine the pulse field gel electrophoresis patterns as well as antimicrobial susceptibility profiles of the isolates; (ii) to examine and characterize the isolates' ability to withstand synthetic gastric juice acid challenge; (iii) to determine the isolates' ability to infect differentiated Caco-2 cells; and (iv) to examine the biofilm formation ability of the isolates.

## 2. Materials and methods

### 2.1. Bacterial strains and growth media

During the years 2000–2003, approximately 3000 food samples collected in Florida using a standard randomized protocol were screened for *Salmonella* (USDA–FSIS, October 01, 2004). Sixteen *Salmonella* isolates from 11 different samples were identified using conventional microbiological criteria (Table 1). All strains were maintained at −70 °C and streaked on LB agar medium prior to use. Single colony obtained after overnight growth at 37 °C was used to inoculate various growth media such as LB-MOPS (morpholinepropanesulfonic acid, 100 mM, pH 8.0) or LB-MES (morpholineethanesulfonic acid, 100 mM, pH 5.5), or minimal E medium with 0.4% glucose. Liquid cultures were grown in shaker–incubator with 220 rpm, at 37 °C for 20 h.

### 2.2. Serotyping and Pulsed-field gel electrophoresis (PFGE)

All isolates were further confirmed as *Salmonella* using VITEK Gram-negative identification cards (BioMerieux Inc., Hazelwood, MO) following the manufacturer's instructions. *Salmonella* isolates were further serotyped by Kauffman–White classification scheme (Brenner, 1998) for O and H antigens using either commercially available Difco (Becton, Dickinson and Company, Sparks, MD) or CDC (Atlanta, GA) antisera.

Pulsed-field gel electrophoresis was performed according to the protocol developed by the Centers for Disease Control and Prevention (CDC) (Centers for Disease Control and Prevention, 1998). PFGE results were analyzed using the BioNumerics Software (Applied-Maths, Kortrijk, Belgium); banding patterns were compared using Dice coefficients with a 1.5% band position tolerance.

### 2.3. Antimicrobial susceptibility testing

Antimicrobial minimum inhibitory concentrations (MIC) were determined using the Sensititre automated antimicrobial susceptibility system in accordance with the manufacturer's instructions (Trek Diagnostic Systems, Cleveland, OH) and the Clinical and Laboratory Standards (CLSI) standards (Clinical and Laboratory Standards Institute, 2002). Results were interpreted in accordance with interpretive criteria provided by CLSI for those antimicrobial agents for whom CLSI had interpretive criteria. (Clinical and Laboratory Standards Institute, 2006). For those antimicrobial agents for whom there were no CLSI interpretive criteria (nalidixic acid and streptomycin) the interpretive criteria used by the National Antimicrobial Resistance Monitoring System (NARMS) were used. The following antimicrobials were tested: amikacin, amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. *Escherichia coli* ATCC 25922 and ATCC 35218, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control organisms in antimicrobial MIC determinations.

### 2.4. Caco-2 invasion assay

The human Caco-2 intestinal cell line was obtained from the ATCC (Manassas, VA). The tissue culture cells were cultivated at 37 °C in a 94% air/ 5%$CO_2$ atmosphere in MEM supplemented with Earle's salts, 20% Fetal bovine serum, 4 mM L-glutamine, 0.1 mM nonessential amino acids, 100 units/ml penicillin, 100 µg/ml streptomycin, and amphotericin B 2.5 µg/ml. All cell culture media and supplements were obtained from GIBCO (Invitrogen, or Quality Biologicals, Inc., Gaithersburg, MD). Caco-2 cells were maintained as confluent monolayers for four weeks prior to use to allow the cells to differentiate prior to use.

The invasion assays were performed essentially as described earlier by Huang et al. (1998) and as modified by Knodler et al. (2002). All assays were conducted in quadruplicate and independently repeated at least twice. Results are expressed as an average of the replicate experiments (+/− represent standard deviation of mean values). Recovery data percentages were calculated and analyzed as described

**Table 1**
Acid-tolerance characteristic of *Salmonella* spp. isolates

| Strain designation | Date of isolation | Source | Acid-tolerance characteristics (% survival after synthetic gastric juice challenge at pH 3.0, 2 h at 37 °C)[a,b] | |
|---|---|---|---|---|
| | | | Inducible acid-tolerance | Constitutive acid-tolerance |
| SL1344, Typhimurium | NA | NA | 28.8±0.4 (B) | 3.1±1.5 (A) |
| LT2, Typhimurium | NA | NA | 4.7±1.0 (C) | 0.01±0.01 (D) |
| 92 | 6/26/2000 | Paprika | 32.0±4.0 (B) | 0.28±0.05 (C) |
| 93 | 6/27/2000 | Irrigation water | 31.0±3.7 (B) | 0.22±0.12 (C) |
| 94 | 7/14/2000 | Paprika (raw imported material) | 28.5±1.8 (B) | 0.88±0.16 (B) |
| 95 | 9/25/2000 | Alligator meat | 39.7±6.6 (B) | 3.3±0.56 (A) |
| 96 | 9/26/2000 | Processed catfish | 40.7±0.67 (B) | 0.32±0.09 (C) |
| 97 | 11/29/2000 | Organic legume mix | 65.8±6.8 (A) | 1.1±0.57 (B) |
| 98 | 1/12/2001 | Paprika | 27.2±2.32 (B) | 0.28±0.01 (C) |
| 99 | 1/24/2001 | Processed catfish | 39.2±9.2 (B) | 0.44±0.06 (B,C) |
| 100 | 1/25/2001 | Alligator meat | 30.9±9.6 (B) | 0.07±03 (D) |
| 101 | 1/8/2002 | Spices | 24.5±1.6 (B) | 0.02±0.02 (D) |
| 102 | 3/19/2002 | Sesame seed alonjoli | 0.26±0.09 (D) | 0.02±0.01 (D) |
| 103 | 4/2/2002 | Dry white cheese | 37.2±6.4 (B) | 0.04±0.01 (D) |
| 104 | 4/12/2002 | Sage Spice | 25.7±3.2 (B) | 0.06±0.04 (D) |
| 105 | 6/27/2003 | Ground cumin | 29.2±0.32 (B) | 0.34±0.08 (C) |
| 106 | 7/14/2003 | Ground cumin | 27.9±9.1 (B) | 0.02±0.01 (D) |
| 107 | 12/3/2002 | Fresh Squeezed orange juice | 26.9±3.9 (B) | 1.4±0.21 (B) |

[a]Mean values ($n=3$) in each column that are not followed by the same letter in the parenthesis indicate significant ($P<0.05$) differences. ± denotes standard deviation of mean.
[b]Inducible acid-tolerance was measured by subjecting cells grown in mild acidic conditions for 24 h (LB-MES, pH 5.5) to synthetic gastric juice challenge and constitutive acid-tolerance was measure by subjecting cells grown in LB-MOPS (pH 8.0) to synthetic gastric juice challenge.

previously (Bhagwat et al., 2006). For all statistical analyses, SigmaStat 3.0 software (Ashburn, VA) was used. Data were analyzed by one-way ANOVA test to determine statistical differences between means of treatments.

### 2.5. Acid-tolerance analysis

To examine the phenotypic expression of constitutive and acid-induced acid-tolerance systems of *Salmonella* isolates, stationary phase cells (over night grown cultures) were diluted directly from the growth media (1:200) to synthetic gastric juice (pH 3.0). To measure constitutive acid-tolerance cells were grown in LB-MOPS (100 mM, pH 8.0) and cells were cultured in LB-MES (100 mM, pH 5.5) to measure inducible acid-tolerance. The synthetic gastric juice was pre-warmed to 37 °C and the pH was adjusted to 3.0 with 6 N HCl. The composition of the synthetic gastric juice was 8.3 g proteose–peptone, 3.5 g glucose, 2.05 g NaCl, 0.6 g $KH_2PO_4$, 0.11 g $CaCl_2$, 0.37 g KCl, 0.05 g porcine bile, 0.1 g lysozyme, 13.3 mg pepsin and Millipore-filtered water 1 L, pH was adjusted to 2.0 with 6 N HCl. (Beumer et al., 1992). Initial viable counts were determined immediately by diluting cells in phosphate buffered saline (50 mM, pH 7.2) and plating on LB agar. Samples were mixed one more time at the end of 2 h acid-challenge incubation before taking aliquots for determining viable cell counts. At least three replicates were included for each acid-challenge assay and data from all replicates was included to plot the survival percentages. To avoid cell density-dependent artifacts (Cui et al., 2001), the population of cells at the beginning of the acid-challenge was maintained at ~0.5 to $1.5\times10^7$ cells/ml (final population). Viable counts were determined after acid-challenge by diluting cells in phosphate buffered saline (PBS) (50 mM, pH 7.2) and plating immediately on LB agar.

### 2.6. Biofilm formation

Biofilm formation was measured as described by O'Toole and Kolter (1998). Briefly, the overnight grown cultures in LB broth were diluted 1:10,000 in fresh media and placed in sterile polystyrene micro plates at 100 µl per well and incubated for 24 h static at 30 °C for use in biofilm studies. $A_{600}$ was measured using micro plate spectrophotometer (BioTek Instruments, Inc.; Winooski, VT) before proceeding for wash and crystal violet staining. The average absorbance of eight control wells (containing uninoculated growth medium) was subtracted from each sample well to determine the amount of biofilm present.

## 3. Results and discussion

### 3.1. Salmonella serotypes, genetic relatedness and antimicrobial resistance phenotypes

From January, 2000 to December, 2003, *Salmonella* were cultured from various foods, spices and water samples collected in Florida using a standard randomized protocol and characterized following the procedure recommended by the USDA/FSIS (http://www.doacs.state.fl.us/fs/foodlab.html). Samples that tested positive for *Salmonella* included meat from alligator ($n$=2) and catfish ($n$=2), various spices ($n$=8) and orange juice, organic legume mix, dry white cheese and irrigation water (one isolate each, $n$=4) (Table 1). Among the 16 isolates, 15 different serotypes were identified, including S. Anatum, S. Baidon, S. Caracase, S. Cubana, S. Give, S. I 13,23,d-, S. Isangi, S. Montevideo, S. Muenchen, S. Newport, S. Onderstepoort, S. Senftenberg, S. Teko, S.Wandsbek and S. Weltevideo. PFGE showed that all 16 *Salmonella* isolates were genetically diverse with a similarity index ranging from 46% to 72% by two enzymes analysis (Fig. 1).

We compared the PFGE patterns to those in the US PulseNet National database, eight out of 16 patterns were indistinguishable from XbaI patterns of strains from certain human clinical samples or foods (Fig. 2). This may be an indication that the isolates in this study could potentially cause human salmonellosis. For example, serotype Anatum (strain 100) obtained from alligator meat was indistinguishable in its PFGE pattern when compared with *Salmonella* isolates obtained from cumin seeds (from India) and human stool, blood or urine samples from Florida, Georgia, Indiana, New Jersey, and New York. Likewise, serotype Muenchen (strain 107) isolated from orange juice in this study had identical PFGE pattern with strains obtained of human origin from New York and South Carolina. We are not aware of any epidemiological links among isolates from the different regions. Since PFGE data are best interpreted in the proper epidemiological connection (Barrett et al., 2006), we are unable to comment on link if any, between these food isolates and human isolates. However, identical PFGE patterns generated from human and food isolates may indicated that these food isolates may have potential to cause human diseases. It could also possible that movement of foodstuffs, animal or people may have been involved in the spread of these strains between countries as suggested earlier (Liebana et al., 2002; Whittam and Bergholz, 2007).

All isolates were quite susceptible to all 15 antimicrobial agents tested. Two isolates, one *S.* Anatume isolated from alligator (strain 100) showed resistance to tetracycline (MIC breakpoint ≥ 16 µg/ml), and

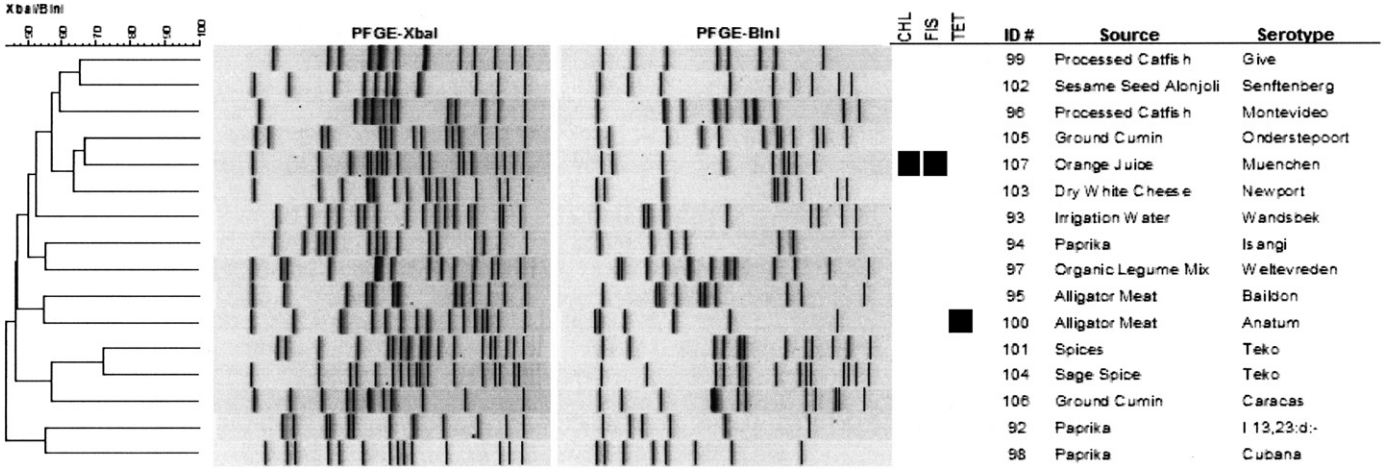
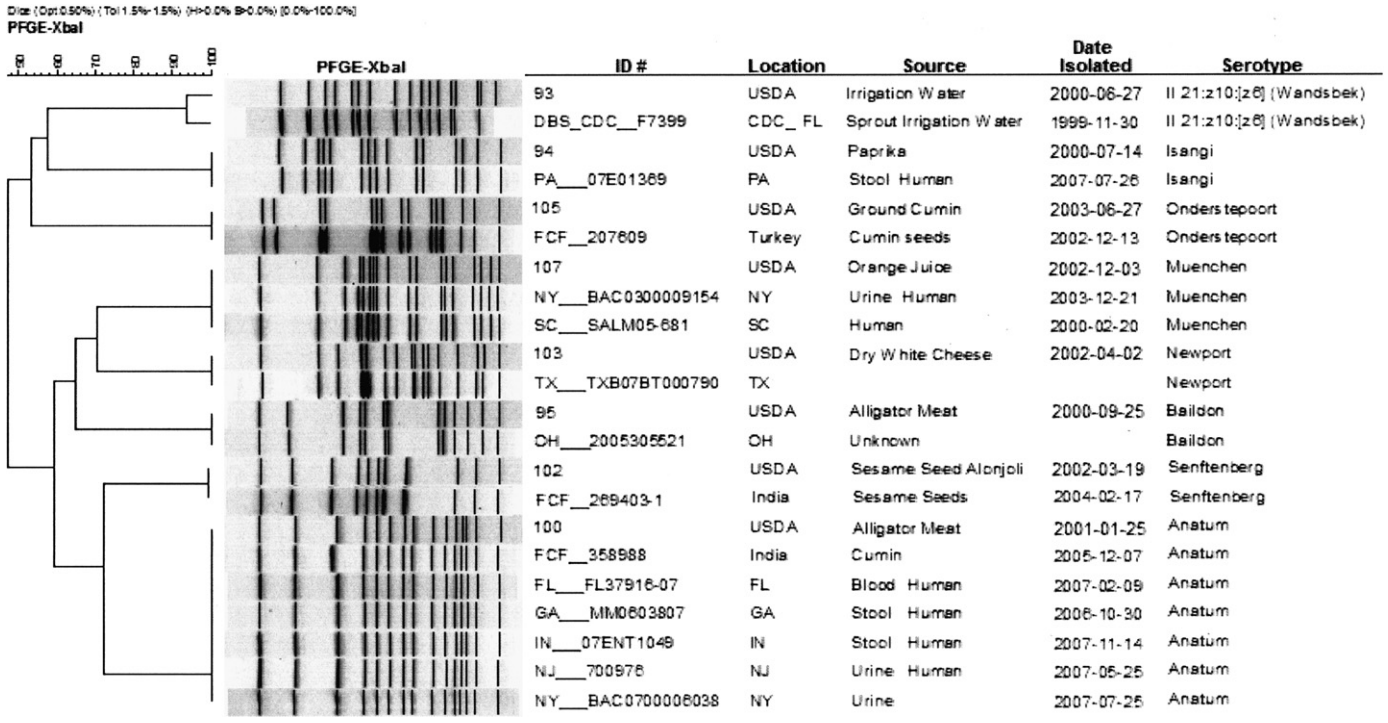



**Fig. 1.** Antibiogram and PFGE patterns of *Salmonella* isolates using restriction endonuclease XbaI and BlnI. A filled square (■) next to strain designation indicates resistance to antimicrobial agent; CHL, chloramphenicol (MIC breakpoint ≥ 32 µg/ml); FIS, sulfisoxazole (MIC breakpoint ≥ 512 µg/ml); TET, tertracycline (MIC breakpoint ≥ 16 µg/ml).




| ID # | Location | Source | Date Isolated | Serotype |
|---|---|---|---|---|
| 93 | USDA | Irrigation Water | 2000-06-27 | II 21:z10:[z6] (Wandsbek) |
| DBS_CDC__F7399 | CDC_ FL | Sprout Irrigation Water | 1999-11-30 | II 21:z10:[z6] (Wandsbek) |
| 94 | USDA | Paprika | 2000-07-14 | Isangi |
| PA___07E01369 | PA | Stool Human | 2007-07-26 | Isangi |
| 105 | USDA | Ground Cumin | 2003-06-27 | Onderstepoort |
| FCF__207609 | Turkey | Cumin seeds | 2002-12-13 | Onderstepoort |
| 107 | USDA | Orange Juice | 2002-12-03 | Muenchen |
| NY___BAC0300009154 | NY | Urine Human | 2003-12-21 | Muenchen |
| SC___SALM05-681 | SC | Human | 2000-02-20 | Muenchen |
| 103 | USDA | Dry White Cheese | 2002-04-02 | Newport |
| TX___TXB07BT000790 | TX | | | Newport |
| 95 | USDA | Alligator Meat | 2000-09-25 | Baildon |
| OH___2005305521 | OH | Unknown | | Baildon |
| 102 | USDA | Sesame Seed Alonjoli | 2002-03-19 | Senftenberg |
| FCF__269403-1 | India | Sesame Seeds | 2004-02-17 | Senftenberg |
| 100 | USDA | Alligator Meat | 2001-01-25 | Anatum |
| FCF__358988 | India | Cumin | 2005-12-07 | Anatum |
| FL___FL37916-07 | FL | Blood Human | 2007-02-09 | Anatum |
| GA___MM0603807 | GA | Stool Human | 2006-10-30 | Anatum |
| IN___07ENT1049 | IN | Stool Human | 2007-11-14 | Anatum |
| NJ___700976 | NJ | Urine Human | 2007-05-25 | Anatum |
| NY___BAC0700006038 | NY | Urine | 2007-07-25 | Anatum |

**Fig. 2.** Comparison of XbaI PFGE patterns of *Salmonella* isolates from current study to human and food isolates from the National PulseNet database at CDC through FDA/CVM PulseNet connection. ID numbers and geographic origin are from the CDC database.

another isolate S. Muenchen obtained from orange juice (strain 107) showed resistance to chloramphenicol (MIC breakpoint ≥32 μg/ml) and sulfisoxazole (MIC breakpoint≥512 μg/ml). *Salmonella* isolates with identical PFGE pattern to that of strains 100 and 107 were also reported from likely human infections (see above). Further studies will be required to establish if *Salmonella* isolates in the PulseNet collection also carry identical antimicrobial resistance pattern. Recently, the National Antimicrobial Resistance Monitoring System (NARMS) posted its report on human clinical isolates obtained in 2004 on the internet (CDC, 2007). The data indicated increase in resistance to nalidixic acid (0.4% in 1996 to 2.6% in 2004) and ceftiofur (0.2% in 1996 to 3.4% in 2004). However, most of the increased resistance was attributed to serotypes Entritidis, Newport and Heidelberg (Gerner-Smidt and Whichard, 2007). In the present study, two out of 16 strains (i.e., strain 100 and 107) were resistant to antimicrobial agent(s) and as described above.

### 3.2. Inducible and constitutive acid-tolerance of Salmonella isolates

*Salmonella* strains like other enteric human pathogens have the ability to survive passage through the acidic environment of the stomach so that they may colonize the mammalian gastrointestinal tract and cause disease (Foster, 2004; Waterman and Small, 1996). In order to access the strength of constitutive acid-tolerance, cells were grown in LB-MOPS (pH 8.0) media until stationary growth phase and challenged in synthetic gastric juice (pH 3.0). Six out of 16 strains were found defective in constitutive acid-tolerance pathway and survived poorly during the acid-challenge (<0.1% survival) (Table 1). On the other hand only one strain was unable to launch acid-tolerance response even after growth in mildly acidic LB-MES (pH 5.5) media (e.g. strain 102). Acid-tolerance response in *Salmonella* sp. is positively regulated by a transcriptional regulator RpoS. As an indirect measure of RpoS functionality, we determined glycogen levels of *Salmonella* isolates and observed no discernable difference after staining colonies with iodine (data not shown). *Salmonella* has evolved multiple overlapping acid-resistance systems which help to deal with gastric acidity which is of particular relevance during infection of the host. The importance of having multiple acid-resistance systems may be of advantage where a strain defective in constitutive acid-resistance pathway can gain acid-resistance under mildly acidic conditions (i.e., strain 100). The central role of acid resistance and stress can be based on the fact that acid adapted cells are resistant to a variety of other stresses such as heat or oxidative stress while heat or oxygen stressed cells are not resistant to low pH (Rychlik and Barrow, 2005).

### 3.3. Caco-2 cell invasion

Since eight *Salmonella* isolates in this study shared PFGE patterns of *Salmonella* isolates from PulseNet including samples of human origin we set out to determine infection potential for each strain using Caco-2 tissue culture assay. Invasion of differentiated Caco-2 cells is often used as a gauge of virulence and infection potential of *Salmonella* isolates (Hurley and McCormick, 2003). Average infectivity (% cells internalized) of eight isolates which had no matching PGFE pattern was 0.174, while average infectivity of eight strains which had matching PFGE pattern on PulseNet was 0.277. The difference in the mean values was not great enough to exclude the possibility that the difference is due to random sampling variability and was not statistically significant ($p=0.33$). Out of six isolates that were defective in constitutive acid-tolerance response, four isolates belonged to serotypes Anatum, Teko (two isolates), and Senftenberg and these isolates infected Caco-2 cells poorly. In Caco-2 cell invasion assay only 0.01% to 0.05% cells were internalized. The serotype Senftenberg strain was defective in both constitutive- and inducible-acid-tolerance (e.g., strain 102, Table 1) and showed reduced Caco-2 cells invasion (0.01% cells internalized). Considering all 16 isolates there was weak correlation observed ($r=0.576$) between Caco-2 cell infectivity and constitutive acid-tolerance ability of *Salmonella* isolates (Fig. 3a). Strain 95 which exhibited highest level of constitutive acid-tolerance (3.3% survival after acid challenge, Table 1) and not considering this the $r$ value dropped to 0.245 (data not shown). Caco-2 cells are derived from human intestinal epithelial tissue and this model has

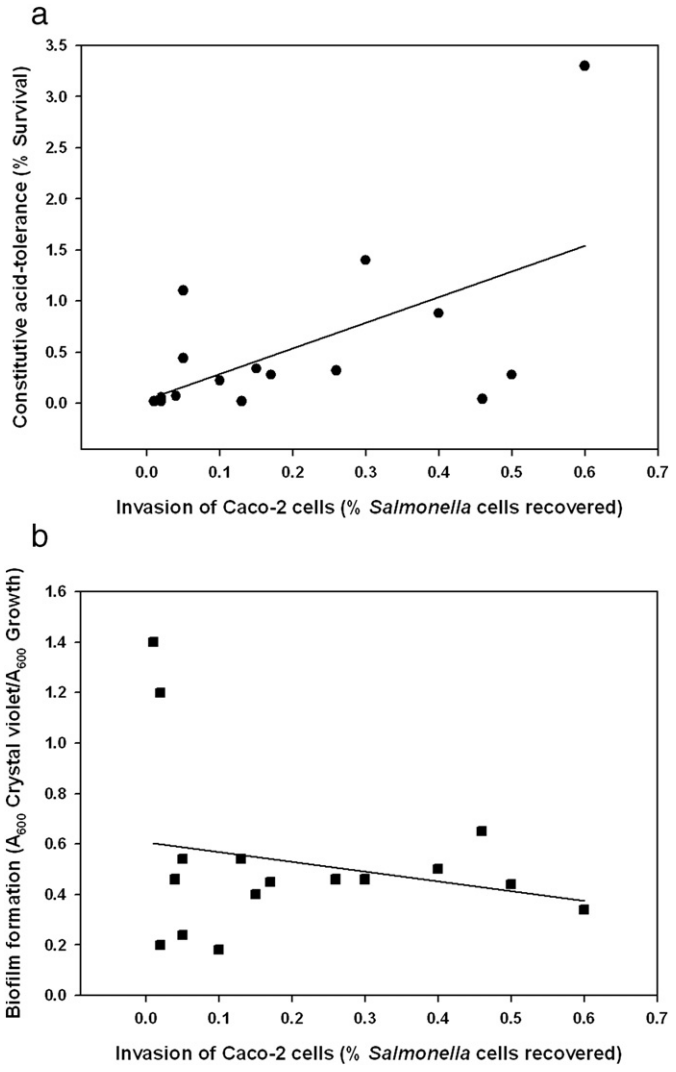



**Fig. 3.** Biofilm formation, Caco-2 cell invasion and constitutive acid-tolerance of *Salmonella* isolates. (a) Constitutive acid-tolerance values (% survival, ●) after subjecting cells which were grown on LB-MOPS (pH 8.0) to synthetic gastric juice shock at pH 3.0 for 2 h. Caco-2 cell invasion was measure as % cells recovered after allowing 4 h for internalization (*x*-axis). Correlation value (*r*) was calculated using a SigmaPlot software version 10.0. (b) Biofilm formation (■) in microtitre plates was measured after staining the washed wells with crystal violet and normalizing the value with growth (turbidity at A600) measured before washing the wells.

served as an extremely useful tool in evaluating molecular mechanisms that underlie pathogenic disease processes. However some heterogeneity in the mechanism of *Salmonella* invasion for various host-cell models has been reported (Hurley and McCormick, 2003). In the past various animal models have been used to determine differences in virulence and invasiveness of various strains. However there is a need to be able to differentiate between virulent and avirulent strains of *Salmonella* without necessarily resorting to the use of animal models (Humphrey et al., 1996), such as in vitro glass adherence test or biofilm formation (see below) (Solano et al., 1998). Further studies are necessary to determine the relationship between virulence and Caco-2 cells invasion which has been reported earlier (Solano et al., 2001).

### 3.4. Biofilm formation

It was proposed that adherence to glass surface may mimic signal to trigger transcription of virulence genes in bacteria and hence may be used to discriminate *Salmonella* strains for their virulence (Solano et al., 1998). We were unable to detect adherence to glass surface in any of the *Salmonella* isolates (data not shown). Nonetheless, attachment of microorganisms and the subsequent development of biofilms in food processing environments are potential sources of contamination and may lead to transmission of diseases (Agle, 2007). Therefore we determined biofilm formation ability using standard microtitre plate assay. All 16 strains formed biofilms (expressed as a ratio of $A_{600}$ after crystal violet staining per $A_{600}$ growth turbidity) with median value of 0.46 and average of 0.53 ± 0.32 (+/− denotes standard deviation of the mean value). One of the serotype Teko isolate (strain 104), and serotype Senftenberg isolate (strain 102) were most prolific in biofilm formation (1.23 ± 0.25 and 1.4 ± 0.21, respectively) and were significantly different than the rest of the strains ($p = 0.017$). The two isolates of Teko serotype differed significantly in their ability to form biofilm as the second isolate of Teko serotype (strain 101) was comparatively weaker in biofilm formation (0.21 ± 0.09). Serotype Anatum (strain 100) and Muenchen (strain 107) which had most hits in PulseNet database formed much less biofilm compared to strains 102 and 104 (0.43 ± 0.18 and 0.48 ± 0.21 respectively). Lastly, we compared biofilm formation ability with Caco-2 cell invasion (Fig. 3b). Strains 102 and 104, although formed high amounts of biofilm they infected Caco-2 cells poorly (0.01% and 0.02% internalization respectively). In general, no relationship was evident among 16 *Salmonella* strains between biofilm formation and Caco-2 cell invasion ($r = 0.230$) and even after exclusion of strains 102 and 104, $r$ was 0.332 (data not shown).

In summary, the study showed that PFGE remains a widely used molecular tool to determine the diversity among *Salmonella* isolates. Although no outbreaks were reported from the naturally contaminated foods that were examined here, eight out of 16 PFGE patterns had a match in the PulseNet database suggesting a possibility that isolates in this study have the potential to cause human illness. The study also supports the notion that some of the *Salmonella* clones could be widespread (Liebana et al., 2002; Whittam and Bergholz, 2007) and occur in several countries and are likely spread as a result of the movement of foodstuffs, animals, or people.

## Acknowledgements

We would like to thank Jason Abbott, Sherry Ayers, Sharon Friedman, and Althea Glenn for excellent technical assistance.

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